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Review

Stem cell biology — a never ending quest for understanding*

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Stem cells (SC) research is an important part of biotechnology that could lead to the development of new therapeutic strategies. A lot of effort has been put to understand biology of the stem cells and to find genes and subsequently proteins that are responsible for their proliferation, self-renewal and differentiation. Different cytokines and growth factors has been used to expand stem cells, but no combination of these factors was identified that could effectively expand the most primitive stem cells. Recently, however, genes and receptors responsible for SC proliferation and differentiation have been described. Ligands for these receptors or these genes themselves are being already used for *ex vivo* expansion of stem cells and the first data are very promising. New markers, such as CXCR4 and CD133, have been discovered and shown to be present on surface of hematopoietic stem cells. The same markers were recently also found to be expressed on neuronal-, hepatic- or skeletal muscle-stem cells. By employing these markers several laboratories are trying to isolate stem cells for potential clinical use. New characteristics of stem cells such as transdifferentiation and cell fusion have been described. Our team has identified a population of tissue committed stem cells (TCSC). These cells are present in a bone marrow and in other tissues and they can differentiate into several cell types including cardiac, neural and liver cells.

Keywords: stem cells, plasticity, TCSC

Stem cells biology is one of the venues of today biomedical research. It is a very promising field with a lot of potential to generate new therapies. Especially in the last decade several important discoveries have been made that shed new light onto the biology of stem cells. But at the same time this new knowledge has raised a lot of new questions and controversies.

Stem cells are very rare cells with two major features; self-renewal and an ability to differentiate into mature cells (Weissman, 2000). Self-renewal is a process during which a stem cell can divide symmetrically and give rise to two daughter stem cells or divide asymmetrically and give rise to one stem cell and one more mature cell. In the first scenario the number of SC is increased, an important feature for stem cells regeneration. In the second scenario SC number is maintained as it happens in steady state conditions. There are different types of stem cells; i) most primitive totipotent SC — the zygote that is able to produce the embryo and placenta, ii) pluripotent SC — embryonic stem cells that give rise only to the embryo, iii) multipotent SC — stem cells that give rise to three germ layers, and iv) tissue committed stem cells (TCSC) that give rise to cells building particular tissues (Lemoli *et al.*, 2005).

Until recently only few tissues were recognized to contain stem cells. But this perspective has changed due to intensive research into this subject. We know now that probably all tissues contain a population of SC that are responsible for their growth and regeneration. Numerous organs have been shown to contain specific stem cells: brain tissue — neuronal stem cells (Eriksson *et al.*, 1998; Johansson *et al.*, 1999), liver tissue — oval stem cells (Alison *et al.*, 1996; 2004) or heart tissue — cardi-

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Abbreviations: ECM, extracellular matrix; HSC, hematopoietic stem cells; SC, stem cells; SDF-1, stromal derived factor-1; TCSC, tissue committed stem cells.

ac stem cells (Beltrami *et al.*, 2003; Laugwitz *et al.*, 2005), to mentioned just a few examples. The best studied are hematopoietic stem cells (HSC) that give rise to all blood cell lineages, both of myeloid and lymphoid origin.

In this review we would like to summarize recent advances made in stem cell biology, particularly in the ability to expand them, the new ways to isolate stem cells and also to discuss some controversies about stem cell behavior and features.

NUMBER IS THE PROBLEM

Stem cells from the different tissues present great potential in cellular therapies and in our quest for longevity. Unfortunately, in most of the tissues, there are not enough of them to fulfill these expectations. HSC are the best known stem cells and they are already used routinely in hematology and transplantation (Kondo *et al.*, 2003). We collect them from different sources such as bone marrow, peripheral blood and cord blood (Kondo *et al.*, 2003; Ballen, 2005). Despite these multiple sources quite often we cannot obtain a sufficient number of HSC for treatment, especially when cord blood is used (Ballen, 2005). Because of that since their discoveries there has been a growing number of different approaches to increasing their number *in vitro*. Cytokines that are present in the bone marrow and stimulate proliferation of hematopoietic cells were first used in attempts to increase the number of HSC in cultures. The goal was that they would induce proliferation but not differentiation of stem cells (Luskey *et al.*, 1992; Bryder & Jacobsen, 2000). Unfortunately, they did not really succeed and after the early enthusiasm for some of the studies, generally they were non-reproducible or the increase was only few-fold.

Bone marrow consists of different cell types, the extracellular matrix (ECM) and soluble factors, mainly cytokines and growth factors (Moore, 2004). Non-hematopoietic cells present in the bone marrow include stromal cells, endothelial cells and osteoblasts that together with ECM form so called "niches" where stem cells reside. Because bone marrow supports hematopoietic cell proliferation and differentiation in the steady state, it seems logical that cells present in the bone marrow should also support the growth of stem cells. This observation has led to the establishment of feeder layer cells derived from bone marrow stromal cells. These cells are able to promote proliferation and differentiation of HSC in long-term cultures (Dexter *et al.*, 1977; Sutherland *et al.*, 1989). Recently, in order to further increase the SC-promoting potential of feeder layer cells, genetic modification has been introduced. One of the approaches is based on introduction of genes that inhibit differentiation of target cells. The Notch pathway, which controls different cellular processes including proliferation, differentiation or apotosis, is also responsible for maintaining various cell types in undifferentiated state (Maillard *et al.*, 2005; Karanu *et al.*, 2000). Its ligands (Jagged-1, Delta) are expressed in the bone marrow milieu by osteoblasts, stromal cells and endothelial cells (Calvi *et al.*, 2003). Forced upregulation of these molecules in feeder cells and HSC might further promote the expansion of stem cells without their differentiation (Zhang *et al.*, 2003).

Another way to go is to directly modified stem cells. Based on our understanding of the molecular pathways responsible for stem cells self-renewal and proliferation as well as discoveries of new genes that control stem cell proliferation and differentiation, new approaches have arisen. One of them uses a member of the *HOX* gene family of transcription factors, *HOXB4*. *HOX* genes are expressed during early development and govern various processes including body-part patterning (Hombria & Lovegrove, 2003). *HOXB4* has been shown to increase the self-renewal potential of HSC. Bone marrow of mice transplanted with HSC virally modified to express *HOXB4* had a comparable number of HSC to the bone marrow of non-transplanted mice. It was in striking contrast to control mice that regenerated only 5–10% of HSC in bone marrow after transplantation (Sauvageau *et al.*, 1995; Antonchuk *et al.*, 2002). Importantly, number of HSC in *HOXB4* mice did not increase over physiological limit and homeostasis was maintained. The expansion of *HOXB4* positive stem cells was also observed *in vitro* (Sauvageau *et al.*, 1995). These experiments showed that controlled manipulation of gene expression by stem cells could lead to development of new therapeutics strategies.

HOW TO ISOLATE STEM CELLS?

An important step in order to study the biology of stem cells is the development of suitable isolation methods. We have several means of isolating different cell populations including stem cells. Cells can be labeled with antibodies against different cell surface markers. Antibodies can be conjugated with fluorescent dyes or magnetic particles and subsequently sorted using fluorescence activated cell sorting (FACS) or magnetic field. Specific cells can also be isolated according to their adherence to plastic and subsequently expanded in appropriate medium (Rando & Blau, 1994). Metabolic dyes such as Hoechst 33342 or Rhodamin 123 have been used (Goodell *et al.*, 1996; Ratajczak *et al.*, 1998).

All these methods are also used to isolate stem cells from different tissues; unfortunately with only partial success.

The main reason why it is so difficult to purify stem cells is that they are extremely rare. The frequency of hematopoietic stem cells in the bone marrow is 1 per 10^4–10^5 bone marrow cells. The estimated number of heart stem cells varies in recent papers from 0.5% to 500–600 cells among all heart cells (Beltrami *et al.*, 2003; Laugwitz *et al.*, 2005). Only few tissues such as skin or gut contain higher number of stem cells due to their regenerative needs but these cells are not very well defined yet.

The second reason why isolation of SC is so difficult, is that most of the stem cells lack specific cell surface markers. They are "lineage negative". Because of this epidermal stem cells, for example, are isolated according to the level of expression of particular markers (e.g., β_1 and α_6 integrins) and not according to specific antigens expressed on their surface (Jones, 1996; Kaur & Li, 2000). The best characterized of all stem cells, HCS are isolated as $CD34^+$, lin^-, $c\text{-}kit^+$, $Thy\text{-}1^+$, $CD38^-$, but CD34 — the best known HSC marker as well as c-kit and Thy-1 are expressed not only on hematopoietic stem cells, but also on more mature cell populations and some authors showed that some HSC are CD34 negative (Goodell *et al.*, 1997).

Recently, however, new surface markers expressed by stem cells have been discovered. CXCR4, a receptor for α-chemokine SDF-1 (stromal derived factor-1) is one of them. CXCR4 is a seven transmembrane receptor that belongs to a large family of G-protein coupled receptors among which are receptors binding chemokines. It has been first shown to be expressed on mouse HSC (Zou *et al.*, 1998). CXCR4 and SDF-1 knock-out animals had a profound decrease in the number of hematopoietic stem cells in the bone marrow. The same animals had a normal number of early myeloid cells in fetal liver (Nagasawa *et al.*, 1996; Zou *et al.*, 1998). Those data suggested an important role of this receptor-chemokine axis in the homing of HSC from the fetal liver to the bone marrow. This was later confirmed by *in vivo* studies in NOD-SCID mice and human hematopoietic cells (Peled *et al.*, 1999; Kahn *et al.*, 2004). CXCR4 is also expressed on more mature cells of the hematopoietic system and it is especially important for B-cells development (Honczarenko *et al.*, 1999).

CXCR4 was also shown to be expressed by other tissue specific stem cells. Our group showed that CXCR4 is expressed by skeletal muscle satellite cells (Ratajczak *et al.*, 2003). The receptor is functional and is responsible for the migration of satellite cells toward an SDF-1 gradient.

CXCR4 was also found on neuronal stem cells (Reiss *et al.*, 2002). CXCR4 knock out animals exhibited mild developmental defect in the central nervous system due to a migratory problem of SC. Also liver stem cells have been shown to express CXCR4 and the CXCR4-SDF-1 axis seems to play a role in mobilization of oval cells into the site of damage after liver injury (Hatch *et al.*, 2002).

Another marker first discovered in hematopoietic system and subsequently found on the surface of other early cells is CD133, formerly known as AC133 (Yin *et al.*, 1997; Miraglia *et al.*, 1997). It has five transmembrane domains and its function is not known yet. CD133 is present on hematopoietic stem cells and progenitor cell also expressing the CD34 antigen (Yin *et al.*, 1997; Miraglia *et al.*, 1997). It is also a marker for early endothelial cell progenitors and very recently it has been shown to be expressed by neuronal stem cells (Peichev *et al.*, 2000; Padovan *et al.*, 2003).

IS "STEM CELL PLASTICITY" FOR REAL OR IT IS JUST A FLUKE?

The idea of stem cells plasticity arose a few years ago when several investigators showed that cells from one tissue could change their fate and give rise to cells of different type. It has been shown that cells from neuronal tissues can be transformed into hematopoietic cells under stress conditions due to myeloablation (Bjornson *et al.*, 1999). Subsequently bone marrow cells were shown to transform into brain, muscle, liver or kidney cells. At the same time skeletal muscle stem cells gave rise to hematopoietic cells after transplantation *in vivo* (Jackson *et al.*, 1999). This unexpected phenomenon was called transdifferentiation. It could have had a very strong impact on the development of new therapeutics strategies for untreatable diseases. Unfortunately several pieces of data from those early reports could not be reproduced in other laboratories (Kawada & Ogawa, 2001) or the same investigators published that cells they used were in fact of different origin (McKinney-Freeman *et al.*, 2002), and in some instances the observed transdifferentiation was an artifact due to high autofluorescence of the studied cells (Jackson *et al.*, 2004). Cell fusion was also stated as an explanation for the observed stem cell plasticity (Terada *et al.*, 2002). It has been shown that under some circumstances cells of different lineages can fuse with each other and that the new cell can acquire characteristics of one of them. Particularly, it has been noted that myeloid cells, monocytes and macrophages are likely to fuse with other cell types (Camargo *et al.*, 2004). This would partially explain why HSC could so easily "transdifferentiate" into other cell types.

Of course we can not rule out completely the possibility that under special circumstances such as myeloablation or profound tissue damage cells could change their fate and transdifferentiate. But it seems now that this is a rather rare event with no clinical implications.

NEW PLAYERS — TISSUE COMMITTED STEM CELLS

Cell fusion can not explain all the transdifferentiation events observed by different groups. Recently several papers have been published in which cell fusion was carefully evaluated and eventually ruled out (Jang *et al*., 2004; Wurmser *et al*., 2004). Our group proposed a different hypothesis to explain this phenomenon. Bone marrow cells were shown to be the most "plastic" cells in our body. That is why we became interested if maybe the bone marrow contains not only cells of hematopoietic and mesenchymal origin but also cells from other tissues. In a set of very carefully designed experiments we found that bone marrow of both humans and mice contains cells with characteristics of non-bone marrow tissues, so called TCSC (Ratajczak *et al*., 2004; Kucia *et al*., 2004). In the populations of human $CXCR4^+$ $CD34^+$ $CD45^-$ cells and mouse Sca-1^+ $CD45^-$ lin^- cells, cells expressing early skeletal (Myf5, MyoD), cardiac (NKx2.5, GATA4), liver (CK19, α-fetoprotein) or neural (nestin, GAFP) markers were present at both mRNA and protein level (Wojakowski *et al*., 2004; Kucia *et al*., 2004). After isolation some of these cells could be grown *in vitro* toward beating cardiomyocytes or neuron-like looking cells.

During injury such as heart infarct or mobilization with cytokines (G-CSF) these cells are also mobilized into peripheral blood (Wojakowski *et al*., 2004; Kucia *et al*., 2004). The number of TCSC is very low, with the highest number of these cells in young animals (Kucia *et al*., 2005), but with new advances in the field of stem cells expansion, they could be isolated from bone marrow or peripheral blood, expanded *in vitro* and subsequently used in the clinic.

CONCLUSION

Stem cells offer a lot of promise and expectations for developing new cell-based therapeutics. Despite the difficulties in their isolation and *in vitro* culture, tremendous progress has been made during the last several years. These new discoveries will bring stem cells closer to the patients' beds and will give hope to patients suffering from untreatable diseases.

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